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| (51) International Patent Classification ⁴ : A61K 31/725, 31/71, C07H 11/04 | A1 | (11) International Publication Number: WO 90/01938 (43) International Publication Date: 8 March 1990 (08.03.90) |
| (21) International Application Number: PCT/AU89/00350 (22) International Filing Date: 18 August 1989 (18.08.89) (30) Priority data: PI 9942 19 August 1988 (19.08.88) AU (71) Applicant (for all designated States except US): THE AUSTRALIAN NATIONAL UNIVERSITY [AU/AU]; Acton, ACT 2601 (AU). (72) Inventors; and (75) Inventors/Applicants (for US only) : PARISH, Christopher, Richard [AU/AU]; 62 Vasey Crescent, Campbell, ACT 2601 (AU). COWDEN, William, Butler [US/AU]; 56 Urambi Village, Crozier Circuit, Kambah, ACT 2902 (AU). WILLENBORG, David, Otto [US/AU]; 1 Needham Place, Sterling, ACT 2611 (AU). (74) Agents: SLATTERY, John, Michael et al.; Davies & Collision, 1 Little Collins Street, Melbourne, VIC 3000 (AU). | | (81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US. Published <i>With international search report.</i> |
| (54) Title: PHOSPHOSUGAR-BASED ANTI-INFLAMMATORY AND/OR IMMUNOSUPPRESSIVE DRUGS (57) Abstract A method of anti-inflammatory and/or immunosuppressive treatment of an animal or human patient comprises administration to the patient of an effective amount of at least one phosphosugar or derivative thereof, or a phosphosugar-containing oligosaccharide or polysaccharide or derivative thereof. | | |

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**"Phosphosugar-based anti-inflammatory
and/or immunosuppressive drugs"**

10 This invention relates to phosphosugars and
phosphosugar containing compounds that possess
anti-inflammatory and/or immunosuppressive activity, and
in particular it relates to the use of these compounds as
anti-inflammatory and/or immunosuppressive agents in
15 animals and man.

 The lysosomes of cells contain a wide range of
degradative enzymes which play a central role in the entry
of leukocytes into inflammatory sites. Lysosomal enzymes,
produced in the rough endoplasmic reticulum, undergo
20 glycosylation followed by a number of 'trimming' and
phosphorylation reactions resulting in oligosaccharides
rich in mannose-6-phosphate residues (1-3). These
mannose-6-phosphate residues are specific recognition
markers of lysosomal enzymes (3). It is this marker on
25 the enzymes that is recognized by a mannose phosphate
receptor (MPR) which mediates transport of lysosomal
enzymes to lysosomes. This receptor functions not only in
internal transport of lysosomal enzymes but is also
important in their secretory pathway and their expression
30 on cell surfaces (1). Receptor-lysosomal enzyme
interactions have been extensively studied (4-6) and shown
to be inhibited by exogenous mannose-6-phosphate. Work
leading to the present invention has been based on the
hypothesis that mannose-6-phosphate and related

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phosphosugar structures might act as anti-inflammatory agents in vivo, possibly by depleting leukocytes of their lysosomal enzymes although this has not been shown previously.

5 As a result of these investigations, it has now been discovered that certain phosphosugars, notably mannose-6-phosphate and fructose-1-phosphate, are in fact effective anti-inflammatory agents, continuous infusion of the sugars inhibiting experimental allergic
10 encephalomyelitis (EAE), an animal inflammatory disease of the central nervous system resembling multiple sclerosis in humans. Polysaccharides containing D-mannose with phosphate residues have also been found to inhibit EAE.

Phosphosugars, particularly mannose-6-phosphate,
15 have also been found to exhibit an anti-inflammatory effect on passively induced adjuvant arthritis. Adjuvant-induced arthritis in the rat shares a number of features with arthritis in humans, viz. the presence of a proliferative synovitis and subcutaneous nodules, swelling
20 of extremities, and ultimately cartilage and bone erosion. This animal model has been extensively used for detection of anti-inflammatory and immunosuppressive drugs.

Finally, phosphosugars have been found to be effective as an immunosuppressant in preliminary
25 experiments, particularly in controlling the delayed hypersensitivity reaction.

In a first aspect, therefore, the present invention relates to the use of phosphosugars and phosphosugar-containing oligosaccharides and polysaccharides as
30 anti-inflammatory and/or immunosuppressive agents. In this aspect, there is provided a method of anti-inflammatory and/or immunosuppressive treatment of an animal or human patient which comprises administration to the patient of an effective amount of at least one

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phosphosugar or a derivative thereof, or a phosphosugar-containing oligosaccharide or polysaccharide or a derivative thereof.

In another aspect, this invention relates to the use of at least one phosphosugar or phosphosugar-containing oligosaccharide or polysaccharide in the preparation or manufacture of a pharmaceutical or veterinary composition for anti-inflammatory and/or immunosuppressive treatment. In this aspect, there is provided a pharmaceutical or veterinary composition which comprises at least one phosphosugar or a derivative thereof, or a phosphosugar-containing oligosaccharide or polysaccharide or a derivative thereof, together with an acceptable pharmaceutical or veterinary carrier or diluent therefor.

Phosphosugars and phosphosugar-containing oligosaccharides or polysaccharides which may be used in accordance with the present invention comprise both naturally occurring and synthetic compounds containing or comprising phosphosugar residues, that is, sugar residues bearing at least one phosphate moiety. Particularly useful phosphosugars include phosphomannoses, phosphofructoses, phosphogalactoses and phosphoglucoses, while particularly useful oligosaccharides or polysaccharides include polysaccharides containing phosphomannose residues. Presently preferred phosphosugars include mannose-6-phosphate and fructose-1-phosphate. Preferred phosphosugar derivatives are the esters including acetate esters, particularly the 1,2,3,4-tetraacetate of mannose-6-phosphate.

Whilst it is not intended that the present invention should be restricted in any way by a theoretical explanation of the mode of action of the phosphosugars in accordance with the invention, it is presently believed that these active compounds may exert their own

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anti-inflammatory effect, by acting as antagonists or competitive inhibitors of the natural ligand of mannose phosphate receptors (MPR) on cells. Accordingly, the active phosphosugars or phosphosugar-containing oligosaccharides or polysaccharides may include any such compounds which are effective antagonists or competitive inhibitors of the natural ligand of the MPR.

The active anti-inflammatory and/or immunosuppressive agents in accordance with the present invention may be used to treat inflammatory diseases or conditions such as multiple sclerosis and rheumatoid arthritis, as well as in the treatment of the inflammatory process associated with the rejection of organ transplants (since massive mononuclear cell infiltrates are usually associated with acute graft rejection). These active agents may be used alone, in combination with one or more other phosphosugars, or in combination with other known anti-inflammatory or immunosuppressive agents. In particular, compositions of phosphosugars and sulphated polysaccharides with heparanase-inhibitory activity may act synergistically and represent a formulation with potent anti-inflammatory activity. The anti-inflammatory activity of these sulphated polysaccharides is disclosed in detail in International Patent Application No. PCT/AU88/00017.

The anti-inflammatory and/or immunosuppressive activity and use of the phosphosugars in accordance with the present invention is demonstrated in the following Example.

30

EXAMPLE 1 Inhibition of EAE.

In this Example, a number of phosphosugars and one phospho-polysaccharide were tested for their ability to

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inhibit development of EAE in rats. (All phosphosugars tested are commercially available and were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.). Experimental details are included in the footnotes to the Tables setting out the test results.

Table I presents data from an EAE experiment in rats where mannose-6-phosphate, administered to animals via osmotic pumps, totally inhibited development of disease. The data presented in Table II demonstrates that a four fold reduction in the mannose-6-phosphate dose (40 mg/rat/week to 10 mg/rat/week) still resulted in a substantial reduction in disease severity, i.e. the lowest dose of phosphosugar reduced disease severity to 37.7% that of control animals.

Analysis of phosphosugar specificity revealed (Table III) that fructose-1-phosphate was as effective as mannose-6-phosphate at inhibiting disease. Fructose-6-phosphate was also a comparatively effective inhibitor of EAE, whereas galactose-6-phosphate, glucose-6-phosphate and fructose-1,6-diphosphate were partially inhibitory. Glucose-1-phosphate and D-mannose apparently had little or no effect on disease progression. These results are displayed graphically in Figure 1. Such phosphosugar specificity closely resembles the monosaccharide specificity of the mannose-6-phosphate receptors on cells (1).

In two separate experiments (Table IV) administration of the D-mannose polysaccharide (mannan) from Saccharomyces cerevisiae, which contains phosphate moieties, totally inhibited EAE, indicating that phosphomannans can inhibit disease.

Histological examination of central nervous system (CNS) tissue from untreated animals with EAE and EAE animals which had been treated with either

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mannose-6-phosphate or mannan containing phosphate moieties, (Table V) revealed that both treatments dramatically inhibited development of CNS lesions. No lesions were detected in mannan-6-phosphate treated 5 animals and a small number of lesions, compared with controls, in mannan treated rats. Such data are consistent with the view that the sugars are inhibiting entry of leukocytes into the CNS.

The first data column in the Tables refers to the 10 number of animals in each group which showed any clinical signs of EAE during the entire course of the experiment. Thus, although 7/10 animals treated with mannan-6-phosphate developed some clinical signs of disease (Table III) the severity of these disease symptoms 15 was extremely mild compared with untreated animals, i.e., <10% disease severity of controls when clinical scores and duration of disease are examined. In this sense, the mannan-6-phosphate data in Tables I and III are almost identical. Similarly, the estimation of disease severity 20 can be used to rank the anti-inflammatory activity of phosphosugars which only partially inhibit disease, e.g., glucose-6-phosphate and fructose-1,6-diphosphate.

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Table I

5 Effect of Mannose-6-Phosphate on Adoptively
 Transferred EAE

| Treatment | No. with EAE/Total | Mean Day Onset | Mean Clinical Score | Mean Length Disease (days) |
|---------------------|-----------------------|----------------------|---------------------------|-------------------------------------|
| Control | 8/8 | 5.2 | 3.0 | 3.5 |
| Mannose-6-phosphate | 0/8 | 0 | 0 | 0 |

Legend to Table I:

EAE induced in Lewis rats with 30×10^6 ConA activated EAE effector cells. Miniosmotic pumps containing phosphosugar
25 were implanted subcutaneously on day 3 after cell transfer. Dose was 40 mg/rat delivered over a 7 day period by 2.0 ml pumps. Clinical EAE was graded according to the following scheme: 0, asymptomatic; 1, flaccid distal half of tail; 2, entire tail flaccid; 3, ataxia, difficulty in righting; 4,
30 hind limb weakness; and 5, hind limb paralysis.

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Table II

5 Effect of Mannose-6-Phosphate Dose on
 Adoptively Transferred EAE

| 10 | Treatment | Dose (mg) | No. with EAE/total | Mean Day Onset | Mean Clinical Score | Mean Length Disease | Disease Severity (%Control) |
|----|-------------------------|--------------|-----------------------|----------------------|---------------------------|---------------------------|-----------------------------------|
| | | | | | | | |
| | Control | - | 4/4 | 4.5 | 3.5 | 4.5 | 100% |
| 15 | Mannose-6- Phosphate | 40 | 1/3 | 5.0 | 0.3 | 0.7 | 1.7% |
| | Mannose-6- Phosphate | 20 | 4/4 | 5.0 | 1.5 | 3.0 | 28.6% |
| 20 | Mannose-6- Phosphate | 10 | 4/4 | 5.0 | 1.8 | 3.3 | 37.7% |

25 Legend to Table II:

Experimental details as in Table I. Mannose-6-phosphate dose represents amount of phosphosugar delivered to rats over a 7 day period via mino-osmotic pumps. "Disease Severity" represents product of mean clinical score and 30 mean length disease.

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Table III

Phosphosugar Specificity of EAE Inhibition

| 5 | Treatment | No.with EAE/Total | Mean Day Onset | Mean Clinical Score | Mean Length Disease (%Control) (days) | Disease Severity |
|----|------------------------------|----------------------|----------------------|---------------------------|---|---------------------|
| 10 | | | | | | |
| 15 | Control | 9/9 | 5.0 | 3.6 | 4.2 | 100% |
| 20 | Mannose-6- phosphate | 7/10 | 6.0 | 0.9 | 1.5 | 8.9% |
| 25 | Fructose-1- phosphate | 3/5 | 5.5 | 1.2 | 1.6 | 12.6% |
| 30 | Fructose-6- phosphate | 4/5 | 6.0 | 1.6 | 2.4 | 25.4% |
| 35 | Galactose-6- phosphate | 5/5 | 5.2 | 2.0 | 3.0 | 40.5% |
| 40 | Glucose-6- phosphate | 5/5 | 5.4 | 2.0 | 3.8 | 50.3% |
| 45 | Fructose-1,6- diphosphate | 5/5 | 5.4 | 2.4 | 3.4 | 54.0% |
| 50 | Glucose-1- phosphate | 5/5 | 5.2 | 3.0 | 3.8 | 75.5% |
| 55 | D-mannose | 5/5 | 5.2 | 2.9 | 4.4 | 84.5% |

40 Legend to Table III:

Experimental details as in Table I. "Disease Severity" represents product of mean clinical score and mean length disease.

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Table IV
Inhibition of Adoptively Transferred EAE
by Yeast Mannan

| | | | | | |
|----|----------------|-----------------------|----------------------|---------------------------|-------------------------------------|
| 5 | <hr/> | | | | |
| | Treatment | No. with EAE/Total | Mean Day Onset | Mean Clinical Score | Mean Length Disease (days) |
| 10 | <hr/> | | | | |
| | <u>Expt. 1</u> | | | | |
| 15 | Control | 5/5 | 4.8 | 3.5 | 4.0 |
| | Yeast mannan | 0/6 | 0 | 0 | 0 |
| | <u>Expt. 2</u> | | | | |
| 20 | Control | 4/4 | 5.0 | 3.1 | 3.7 |
| | Yeast mannan | 0/4 | 0 | 0 | 0 |

25 Legend to Table IV:
Yeast mannan from Saccharomyces cerevisiae (Baker's yeast).
Experimental details as in Table I.

30

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Table V
Histological analysis of EAE Inhibition in Rats
Receiving Mannose-6-Phosphate and Mannan

| 5 | | | |
|-----------------------|----------------------|-------------|-----------------|
| Treatment | No. Sections scanned | No. Lesions | Lesions/section |
| <u>Expt. 1</u> | | | |
| 10 Control 1 | 10 | 110 | 11.0 |
| Control 2 | 8 | 206 | 25.7 |
| Mannose-6-phosphate 1 | 18 | 0 | 0 |
| Mannose-6-phosphate 2 | 15 | 0 | 0 |
| <u>Expt. 2</u> | | | |
| 15 Control 1 | 15 | 284 | 19.0 |
| Control 2 | 12 | 303 | 25.0 |
| Yeast mannan 1 | 18 | 20 | 1.1 |
| Yeast mannan 2 | 15 | 92 | 6.7 |

20

Legend to Table V:

Rats were killed 9 days after cell transfer and sections of the lower thoracic-upper lumbar spinal cord examined for inflammatory lesions. Animals treated as in Table I.

25

EXAMPLE 2 Inhibition of EAE

In further experiments using the EAE model of Example 1, other mannose phosphate-containing compounds were used, including PPME and a pentasaccharide.

30

PPME is the purified high molecular weight, acid-resistant fragment, (polysaccharide core fraction) of the isolated exocellular phosphomannan produced by Pichia holstii (Hansenula holstii) as described by Bretthauer et.al. (7), that contains mannose phosphate residues.

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The pentasaccharide is an isolated monophospho-mannopentaose fragment, 6-phospho-mannose- α (1-3)-{mannose- α -(1-3)}₂-mannose- α -(1-2)-mannose, of the exocellular phosphomannan produced by Pichia holstii 5 (Hansenula holstii) described by Bretthauer et.al. (7).

In these experiments, details of which were as in Table I, the number of cells transferred was 25×10^6 /rat, while the dose of compound administered was 10mg/rat delivered over a 7 day period by mini-osmotic pumps, commencing on day 3 after 10 cell transfer. The results are set out in Table VI.

Table VI

| | <u>Control</u> | <u>PPME</u> | <u>Pentasaccharide</u> |
|-----------|----------------|-------------|------------------------|
| EAE/Total | 5/5 | 3/5 | 1/5 |

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EXAMPLE 3 Suppression of Passive Adjuvant Arthritis

(DA x Lew)F1 rats were immunized with M.butyricum in light mineral oil given in each foot. Ten days later spleens were removed and incubated as single cell suspension tissue culture medium in + 5µg/ml ConA for 75 hrs. Cells were harvested, washed and transferred i.v. at 65×10^6 cells/rat into (DA x Lew)F recipients.

Treated rats were implanted on the day they received cells with miniosmotic pumps which delivered 6mg/rat/day of mannose-6-phosphate for 14 days. Control rats were sham operated. The results are shown in Table VII as % of pre-cell injection foot size. {Average for group; n=4 (mannose-6-phosphate); n=6 (control)}.

15

Table VII

| <u>Day</u> | <u>Mannose-6-Phosphate</u> | <u>Control</u> |
|------------|----------------------------|----------------|
| 4 | 97.3% | 106.4% |
| 6 | 105.8% | 129.7% |
| 7 | 102.8% | 149.7% |
| 20 9 | 108.4% | 148.5% |
| 11 | 107.6% | 184.2% |
| 14 | 117.4% | 220.1% |

25 EXAMPLE 4 Effect on Delayed-Type Hypersensitivity (DTH)

C57Bl mice were sensitised by i.v. injection 10^5 of washed sheep red blood cells. 5 days later they were challenged in the right hind footpad with SRBC. Each mouse was given a 0.25ml injection i/p at the same time of either saline, mannose-6-phosphate or the 1,2,3,4-tetraacetate of mannose-6-phosphate and all injections were repeated a further 6 times at approx. 3½ hour intervals. The dose in each injection of mannose-6-phosphate was 0.15mg and of 1,2,3,4-tetraacetate of mannose-6-phosphate was also 0.15mg. At 24

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hours after challenge the DTH swelling was measured. Mannose-6-phosphate reduced the swelling by 52.5%, and the 1,2,3,4-tetraacetate of mannose-6-phosphate by 91.5%, as compared with the saline controls.

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CLAIMS:

1. A method of anti-inflammatory and/or immunosuppressive treatment of an animal or human patient, which comprises
5 administration to the patient of at least one phosphosugar or a derivative thereof, or a phosphosugar-containing oligosaccharide or polysaccharide or a derivative thereof.
2. A method according to claim 1, wherein said phosphosugar
10 is selected from the group consisting of mannose-6-phosphate, fructose-1-phosphate, fructose-6-phosphate, galactose-6-phosphate, fructose-1,6-diphosphate, glucose-6-phosphate and glucose-1-phosphate.
- 15 3. A method according to claim 2, wherein said phosphosugar is mannose-6- phosphate.
4. A method according to claim 2, wherein said phosphosugar is fructose-1-phosphate.
- 20 5. A method according to claim 1, wherein said phosphosugar derivative comprises an acetate or other ester thereof.
6. A method according to claim 5, wherein said phosphosugar
25 ester is the 1,2,3,4-tetraacetate of mannose-6-phosphate.
7. A method according to claim 1, wherein said phosphosugar-containing oligosaccharide or polysaccharide is an oligosaccharide or polysaccharide containing phosphomannose
30 residues.
8. A method according to claim 7, wherein said oligosaccharide, or polysaccharide is the D-mannose polysaccharide (mannan) from Saccharomyces cerevisiae.

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9. A method according to claim 7, wherein said oligosaccharide or polysaccharide is the purified high molecular weight, acid-resistant fragment (polysaccharide core fraction) of the exocellular phosphomannan produced by Pichia
5 holstii (Hansenula holstii), or an oligosaccharide fragment derived therefrom.

10. A method according to claim 9, wherein said oligosaccharide fragment is the monophosphomannopentaose
10 fragment, 6-phospho-mannose- α (1-3)-{mannose- α -(1-3)}₂-mannose- α -(1-2)-mannose.

11. A method according to claim 1, wherein said treatment comprises treatment of inflammatory disease of the central
15 nervous system.

12. A method according to claim 1, wherein said treatment comprises treatment of arthritis.

20 13. A method according to claim 1, wherein said treatment comprises treatment to control the delayed-type hypersensitivity reaction.

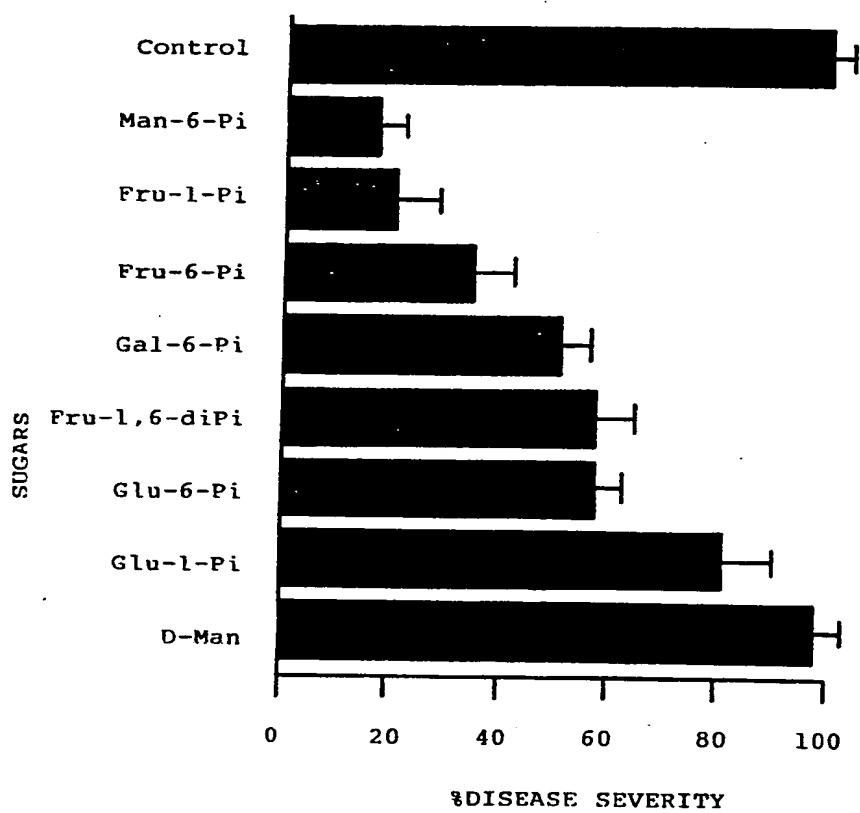
14. A pharmaceutical or veterinary composition for anti-
25 inflammatory and/or immunosuppressive treatment which comprises at least one phosphosugar or derivative thereof, or a phosphosugar-containing oligosaccharide or polysaccharide or a derivative thereof.

30 15. Use of at least one phosphosugar or derivative thereof, or a phosphosugar-containing oligosaccharide or polysaccharide or a derivative thereof for anti-inflammatory or immunosuppressive treatment of an animal or human patient.

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16. Use of at least one phosphosugar or derivative thereof, or a phosphosugar-containing oligosaccharide or polysaccharide or a derivative thereof for the preparation of a medicament for anti-inflammatory and/or immunosuppressive treatment of an animal or human patient.

1 / 1

FIGURE 1

SUBSTITUTE SHEET